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NUTRITION REVIEWS

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CHRONIC PROTEIN MALNUTRITION

At its Third Session in November 1952 the Joint FAO/WHO Expert Nutrition Committee (*WHO Technical Report Series No. 72 (1953)*) composed the following definition of "protein malnutrition": "A state of ill health occurring where diets are habitually poor in protein, while they are more nearly adequate in calories. Clinically, protein malnutrition is most easily recognised when there is a relatively high intake of calories from starchy foods together with an insufficient intake of protein. The concept includes the effects of deficiency in the quantity of protein consumed, of imbalance of amino acids, and deficiency of factors, such as Vitamin B_{12}, commonly found in foods in association with animal protein and concerned with protein metabolism."

The session was held in West Africa immediately after a conference of CCTA (Commission for Technical Cooperation in Africa South of the Sahara, Report of Second Inter-African Conference on Nutrition (1952), *Her Majesty's Stationery Office, London (1954)*) which had been considering the nutrition of mother and child in Africa against the background of kwashiorkor. This African name was first used to denote a clinical syndrome or syndromes resulting from protein malnutrition in the weaning and postweaning stages of early childhood. It is widely prevalent in underprivileged parts of the world both within and without the tropics where customary methods of weaning are based largely or wholly on starchy paps, without reasonable additions of animal or good vegetable protein.

The great majority of the milder cases of kwashiorkor probably correct themselves by greater protein consumption as the child's teeth begin to appear, and there is little if any evidence yet that the damage done to the pancreas, liver, and other organs can not be completely and permanently reversed by sustained good feeding. Throughout the greater part of the world, however, in regions where kwashiorkor and related syndromes occur there is a permanent shortage of protein-rich foods. It is likely, therefore, that diets which would be regarded by American nutritionists as seriously deficient in protein, at least from animal sources, are the permanent lot of millions of people. The question therefore arises whether chronic protein malnutrition, as defined by the Joint Committee, may not be a source of much chronic ill health.

The regions of "endemic kwashiorkor" have recently been mapped by J. F. Brock (Proceedings of the conference on Nutritional Factors and Liver Diseases of the New York Academy of Sciences, *Ann. N. Y. Acad. Sci.* **57,** *696 (1954)*) and it is clear that if all the names listed in Appendix 2 of the Joint Committee's report (Joint FAO/WHO Expert Committee on Nutrition, *loc. cit.*) do in fact represent "syndromes which have the same basic characteristics as kwashiorkor" then this is a problem of great magnitude and importance. If all these regions are regions of "chronic protein malnutrition" then it becomes important to know the extent and nature of resultant ill health.

The Joint FAO/WHO Expert Nutrition Committee in defining "protein malnutrition" was attempting to extend the considerations of the CCTA Conference to include related problems throughout the world. There is little doubt that in this context the term "protein malnutrition" is valuable. It focuses attention on a public health problem of great magnitude, namely, the need for the production and consumption of greater quantities of protein foodstuffs and on the need for milk substitutes in the weaning of children. From the scientific point of view,

however, there has been some tendency to question whether protein malnutrition is an entity in the sense, for example, of the term "hypovitaminosis." The latter term, of course, needs further definition, as for example "hypovitaminosis A," but is nevertheless a useful general term. Those who have criticized the term "protein malnutrition" do so on three counts: (1) that protein is a very indefinite term for a great variety of combinations of amino acids and that it would be better to talk about amino acid deficiency; (2) that protein foodstuffs contain vitamins and minerals as well as amino acids; (3) that calorie intakes are not given due consideration.

In classic kwashiorkor calorie intakes are adequate or nearly adequate, as evidenced by consideration of the caloric content of the starchy diets and by the thick layer of subcutaneous fat which is often found at autopsy. Such cases are well exemplified in the monograph of J. F. Brock and M. Autret (*WHO Monograph Series No. 8 (1952)*), especially in figure 6. In such cases one might question whether the limiting nutrient is simply in the amino acid class or whether deficiency of accessory food factors such as vitamins or trace elements may limit the utilization of quantities of amino acids which would be adequate in the presence of greater quantities of those accessory food factors. This situation might be represented by the analogy of building a brick wall. It is evident that a brick wall can not be built without a certain minimum of bricks. With an insufficiency of plaster it may be necessary to build a 9 inch wall to insure stability. With a good plaster it may be possible to build an equally stable 4½ inch wall with resultant economy of bricks. In this analogy the bricks would represent amino acids and the plaster would represent accessory food factors such as vitamins or trace elements. This hypothesis of protein malnutrition will only be settled by further research. It is obvious, for example, that if kwashiorkor could be cured by a mixture of synthetic amino acids then it might be called amino acid malnutrition. Such a concept, however, may be questioned in that protein foods always contain vitamins and trace elements in addition to amino acids.

Although in the classic syndrome of kwashiorkor calorie needs are adequately supplied by starchy foodstuffs, it is clear that in many parts of the world children showing clinical manifestations of kwashiorkor are also undernourished in respect of calories. The Joint Committee recognized this and discussed both "severe undernutrition in infants" and "intermediate states in which some but not all of the manifestations of kwashiorkor and undernutrition are present." Obviously, if calorie needs are not met then some protein will be wasted in the production of energy and less will be available for tissue building.

Severe undernutrition in infants (marasmus) will result most typically from diets which are qualitatively adequate but deficient in quantity, as occurs in an infant given no supplementary or complementary feeding when the maternal breast yield is failing. But what terms are to be applied to the results of feeding diets in which simultaneously calorie yields and protein/calorie ratios are markedly reduced? Such diets are particularly prevalent in Central and South America and are responsible for many of the cases of "sindrome pluricarencial infantil," a term which was adopted by the Third Conference on Nutrition Problems in Latin America (*FAO Report Series No. 8, Rome, June 1954*) as an American synonym for kwashiorkor. Is the nutrition process which leads to this syndrome also to be called "protein malnutrition"? Such an extension of the term is consistent with the original definition since the Third Joint Committee stated merely that "protein malnutrition is most easily recognized when there is a relatively high intake of calories from starchy foods." It is clear from the context of the succeeding paragraphs that intermediate states were to

be recognized, to which the term "protein malnutrition" might also be applied.

Returning to the geographic significance of protein malnutrition, attention has been devoted to the possible sequelae. Over much of the world area of "endemic kwashiorkor" there is a high incidence of adult liver cirrhosis and hepatoma (Brock, *Ann. N. Y. Acad. Sci., loc. cit.*; C. Berman, "Primary Carcinoma of the Liver," *H. K. Lewis, London (1951)*) and the question has been raised how far these chronic liver diseases can be regarded as sequels either of kwashiorkor, or, more likely, of chronic protein malnutrition.

The evidence has been reviewed elsewhere (Brock, *Ann. N. Y. Acad. Sci., loc. cit.*; J. N. P. Davies, *Ann. Rev. Med.* **3,** *99 (1952)*; J. Gillman and T. Gillman, "Perspectives in Human Malnutrition," *Grune and Stratton, New York (1951)*) and the answer must await further research. In the meantime it can be said that the suggestion accords reasonably with the results of experimental dietary hepatic damage although there are many discrepancies (Brock, *Ann. N. Y. Acad. Sci., loc. cit.*). At least it can be said that a case has been made for investigation. As in the case of kwashiorkor these chronic liver diseases, although concentrated in the tropical belt, are not confined to it. If tropical parasites play a part in the etiology of what is often called "tropical cirrhosis" then that part must often be a minor one, and cirrhosis indistinguishable in its form and in its effects is found in areas free from tropical parasites. Thus the liver cirrhosis which almost invariably underlies hepatoma in the regions of high endemicity, and which has often in the past been attributed to schistosomiasis or liver fluke, is found, together with hepatoma, in young Bantu adults in the Union of South Africa (Gillman and Gillman, *loc. cit.*), far south of the Tropic of Capricorn in areas where the population has never been exposed to these or any other strictly tropical parasites. These populations have, however, lived on diets which in common with diets from the greater part of the tropical world must result in chronic protein malnutrition. There is considerable evidence to suggest that in at least some areas toxic substances contaminating food (*e.g.*, synceio alkaloid) and viruses may play a contributory role in etiology, but that, nevertheless, protein malnutrition is the basic causative factor, and that improvement in diets may be the most important public health measure (Joint FAO/WHO Expert Committee, *loc. cit.*; CCTA Conference, *loc. cit.*; *Ann. N. Y. Acad. Sci., loc. cit.*). If the central position of protein malnutrition in the etiology of the chronic liver diseases of the tropical world is accepted then it becomes possible that protein malnutrition or some other type of malnutrition may be important or even central in the etiology of some diseases of uncertain etiology which are endemic in tropical areas and less commonly encountered in temperate areas. This may be speculation, but it is not idle speculation. The suggestion does not deny the possibility that tropical parasites, viruses, other infections, toxic contaminants in food and even climatic stresses may play an important contributory role. It does, however, imply that improvement in diets may be the most important measure in the control of these diseases.

The diseases of the African (M. Gelfand, "The Sick African," *2d edition, Juta and Co., Cape Town, South Africa (1947)*) and their relation to malnutrition (CCTA Conference; Brock and Autret; Davies; Gillman and Gillman; *loc. cit.*; H. C. Trowell, J. N. P. Davies, and R. F. A. Dean, *Brit. Med. J.* **2,** *796, 798 (1952)*; "Kwashiorkor," *Edward Arnold, London (1954)*) have been well reviewed and consideration of this subject can be used as an example of the effects of tropical malnutrition.

As would be expected if chronic protein malnutrition were a widespread cause of ill health, almost every system of the body is affected by diseases which are seldom if ever encountered in temperate climates and more privileged communities. Some of the more obvious diseases will be mentioned briefly

by way of illustration. Unexplained congestive cardiac failure is prevalent in South Africa and is associated with large hypodynamic, dilated but often not hypertrophied, hearts (A. D. Gillanders, *Brit. Heart J.* **13,** *177 (1951)*). Endomyocardial fibrosis occurs in Uganda and probably other parts of Africa (J. D. Ball, A. W. Williams, and J. N. P. Davies, *Lancet* **I,** *1049 (1954)*, Williams, Ball and Davies, *Tr. Roy. Soc. Trop. Med. Hyg.* **48,** *290 (1954)*). It is probably distinct from the variety described by Gillanders, but its etiology is obscure. Its possible relationship to two other obscure tropical diseases affecting endothelial membranes is worth considering, namely, veno-occlusive disease of Jamaica and idiopathic thrombophlebitis of Rhodesia (A. C. Fisher, M. M. Fisher, and A. C. Lendrum, *J. Path. Bact.* **59,** *405 (1947)*). Tropical ulcer (usually on the lower leg), and tropical pyomyositis (Gelfand, *loc. cit.*), although easily attributed to infection, have never yielded a specific causative microorganism. The same is true of keratoconjunctivitis (C. J. Blumenthal, *South African Med. J.* **24,** *191 (1950)*) in the Bantu people of South Africa right outside the tropics. In the same people young males show a high incidence of gynecomastia and testicular atrophy (Gillman and Gillman, *loc. cit.*), and uterine rupture is common in females in Uganda (CCTA Conference, *loc. cit.*; Davies, *loc. cit.*). Chronic parotid enlargement is prevalent in many parts of the continent. In kwashiorkor there is usually a mild albuminuria and although there is little evidence of renal damage (Davies, *loc. cit.*; Trowell, Davies and Dean, *loc. cit.*), either temporary or persistent, it is interesting that a nephrotic syndrome with a peculiar variety of renal amyloidosis has been recorded in Uganda (A. B. Raper, *East African Med. J.* **30,** *49 (1953)*).

Although protein malnutrition is not a public health problem in the United States, recent knowledge of its frequency and importance elsewhere has acted as a stimulus to experimental work particularly in relation to liver metabolism. There is moreover a need for further critical consideration of human requirements of protein and amino acids, and especially of the extent to which vegetable proteins may safely be substituted for animal proteins in the human dietary.

It would appear that the scientific problems posed by recent concepts of the etiology and pathogenesis of kwashiorkor need to be extended to cover the whole range of the mechanism and effects of "chronic protein malnutrition." Such consideration has recently been given to the subject at a conference in Jamaica in November 1953 sponsored jointly by WHO, FAO and the Josiah Macy Foundation. The report of this conference will appear shortly and is full of scientific interest.

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COMPARISON OF THYROXINE AND TRIIODOTHYRONINE IN MAN

The thyroid gland secretes a thyroid hormone which profoundly affects metabolism (*Nutrition Reviews* **7,** *97 (1949)*). Increased secretion or exogenous administration of the hormone increases the production of energy from food and, as a consequence, usually stimulates appetite and food intake. The thyroid hormone is known to contain 4 atoms of iodine per molecule. Iodide absorbed into the gland is enzymatically oxidized and then combines with tyrosine to form thyroxine which has been considered to be the thyroid hormone (*Ibid.* **8,** *129 (1950)*). Excess thyroid activity leads to the clinical state of hyperthyroidism or thyrotoxicosis, diminished activity to hypothyroidism, myxedema,

or in children to cretinism. The classic work of Kendall, Harrington, Barger, and others led to the identification of the amino acid thyroxine and its synthesis *in vitro*.

The discovery of triiodothyronine in plasma and, under certain circumstances, its greater activity than that of thyroxine has led to the belief that this newly discovered compound might be the form of thyroid hormone active in the peripheral tissues (J. Gross and R. Pitt-Rivers, *Lancet* **I,** *593 (1952)*). This concept is substantiated by three studies by C. M. Blackburn, W. M. McConahey, F. R. Keating, and A. Albert (*J. Clin. Invest.* **33,** *819 (1954)*), Blackburn and Keating (*Ibid.* **33,** *918 (1954)*), and K. Sterling, J. C. Lashof, and E. B. Man (*Ibid.* **33,** *1031 (1954)*).

In the first of these investigations 7 patients with spontaneous myxedema, and 1 in whom hypothyroidism occurred after radioactive iodine therapy, were used for comparing the relative activities of triiodothyronine and thyroxine on the metabolic rate and the serum protein-bound iodine. It was found that a single small dose of triiodothyronine induced a greater total cumulative increase in energy output as measured by oxygen consumption than thyroxine. The reverse was true with larger doses. The action of the triiodothyronine was much more rapid than that of thyroxine and the half-life of action after maximum was much shorter for the former than for the latter. These results are, as the authors say, compatible with the concept that triiodothyronine is the "tissue form of thyroid hormone."

In the second study Blackburn and Keating compared the dose of each of the drugs required to maintain patients with myxedema with a normal metabolic rate ("euthyroid state"). They found that the new material was three to four times as potent as thyroxine.

Sterling and co-workers measured the disappearance in serum radioactivity after the intravenous injection of tracer doses of thyroxine or triiodothyronine labeled with I^{131} in normal individuals (euthyroid). The patients were all either given Lugol's solution (iodine in potassium iodide) or potassium iodide solution for several days prior to study to render ample iodine available to the thyroid so that iodine of the injected hormones after they were metabolized would not be re-utilized. In the 11 individuals studied there was an initial rather rapid decrease in serum radioactivity taking from one to three days, presumably due to distribution of the hormone. Following this the disappearance of the hormones pursued a linear course (on a logarithmic scale) for the fourteen days in which it was observed. By extrapolating this straight line back to zero time and considering the radioactivity of the sample taken at ten minutes after injection as 100 per cent, the "half-life" of the 2 compounds could be determined. The mean half-time for thyroxine was 6.7 and that for triiodothyronine, 2.7 days.

These data confirm the more rapid disappearance of the action of triiodothyronine and thyroxine and are also compatible with the concept that the former may be the "tissue hormone," although, as Sterling and his colleagues point out, the role of triiodothyronine in metabolism is by no means clarified or defined.

PHYSIOLOGIC ADAPTATION IN PREGNANCY

An extensive study on "Physiological Adaptation and Nutritional Status During and After Pregnancy" has been reported by I. G. Macy and co-workers (*J. Nutrition* **52,** *Suppl. 1, pp. 1–92 (1954)*). This study was aimed largely at the establishment of standards: averages and "normal" ranges of variation of six blood components during the

course of uncomplicated pregnancy and following parturition. It was designed also to furnish additional information on individual variability as well as the relation of race, environment, season and dietary habits to some blood constituents which have been useful in the evaluation of nutritional status.

The subjects of the investigation fell into three categories: Group A consisted of 653 women attending a public prenatal clinic and representing the indigent and low-income populations of Detroit. Group B was composed of 164 subjects with moderate incomes from a private prenatal clinic. Group C was composed of 230 private patients almost entirely from middle-class backgrounds with a few wealthy individuals. Groups A and B included both Negro and white subjects in the ratio of 5:1 and 4:1 respectively. Group C did not include Negroes, a fact of some significance in attempting to evaluate racial differences in blood composition. In order to establish the first tentative standards of "normal" changes in blood composition during pregnancy, available data were compiled for individuals satisfying the following criteria for inclusion in the "normal" or "Selected" Group: absence of structural defects or disease, freedom from clinical evidence of malnutrition, absence of serious complications associated with pregnancy and parturition. Of a total of 1064 women in groups A, B and C only 427 qualified for inclusion in the Selected Group. For purposes of comparison repeated blood samples were taken from a group of 48 healthy, nonpregnant white women and from a group of 24 white males. The subjects in these two groups were professional people associated with hospitals or laboratories and were presumably more thoughtful than the average person about their dietary habits.

Extensive information on nutritional status was accumulated showing that the diet ratings of group A (low-income group) were on the average inferior to those of other groups. Quality of prenatal care (length of time under medical care and cooperation in following instructions) was also inferior for group A.

The core of the study, chemical analysis of blood, was carried out on a total of 3766 maternal and 732 infant blood samples. Making use of the micro methods developed by Bessey and Lowry, determinations of hemoglobin, serum protein, ascorbic acid, alkaline phosphatase, vitamin A and carotenoids could be carried out on 0.3 ml. of blood drawn by finger puncture (or heel puncture in the case of the infants).

The scope of this study attests again to the importance of the Bessey-Lowry methods in making possible large-scale investigations. The bulk of the results is presented neatly in a series of six figures. Each figure depicts the results obtained for one of the six chosen blood constituents. It charts the values obtained for the Selected Group from the second month of gestation until several days after parturition. For comparison the results obtained with the 48 nonpregnant white subjects are also shown. Finally the values obtained on the blood samples taken from the newborn infants are pictured. In each figure are shown the median and the upper and lower ranges after 10 per cent of the results at either extreme had been eliminated.

Hemoglobin levels of the pregnant women showed perhaps a slight tendency to decline during gestation, the values for Negroes tending to run somewhat lower than those of the white members of the group. Infant hemoglobin levels were much higher than maternal levels, with a median near 19 g. per cent. Ascorbic acid values also showed a tendency to decrease during gestation with the median for the Negro group running very much lower than that for the white. The median for the white group remained well above 1 mg. per cent, an indication that the ascorbic acid intake of this group was commendably satisfactory. The median for the Negro members of the group dropped to as low as 0.54 mg. per cent. An interesting observation was the

decline in ascorbic acid levels following parturition, which was particularly marked in the white group. Whether this represents the reflection of the stress of delivery or is the result of hospital diet cannot be assessed from the data presented. The ascorbic acid levels of the infants showed medians well above 1 mg. per cent.

Serum protein levels of the pregnant women were initially lower than those of the nonpregnant control group. The levels declined slightly between the second month of pregnancy and parturition, the levels of the Negro group being consistently higher than those of the white group. Serum proteins of the newborn infants were lower on the average than those of the mothers. Serum vitamin A declined in the Negro group but remained about constant in the white group. Again the offspring showed lower levels than the mothers. Serum carotenoids tended to increase slightly during gestation, but the levels in the infants were very low when compared with those of the mothers. In contrast, serum alkaline phosphatase increased dramatically during the latter part of gestation, with the infants' levels as high as the final levels attained by the mothers. The increased phosphatase content of maternal blood coincides with the initiation of calcium deposition in the fetal bone.

The interpretation of the observed birth weights presented a problem of some general interest. These investigators had adopted as one criterion of delivery at term a birth weight of at least 2500 g. According to this criterion the incidence of premature births was found to be higher for the Negro subjects than for the whites. If, on the other hand, separate standards are accepted for the two racial groups—a lower limit of 2500 g. for white infants and 2300 g. for Negro infants—the difference in incidence of prematurity tends to disappear. That such a dual standard may be justified is suggested by the finding that the median birth weight of white infants in the Selected Group was 3307 g., while that of the Negro infants was 3089 g. However, it is still uncertain to what extent the mothers' nutritional status may influence the infants' birth weights. The diet ratings of the Negro women in the Selected Group were appreciably lower than those of the white women of this group. The relation of maternal dietary habits to the course of pregnancy and the health of the offspring has, of course, been studied at length by Ebbs, Burke and others (*see Nutrition Reviews* **1,** *276, 386 (1943)*; **3,** *192 (1945)*; **4,** *175 (1946)*; **12,** *104 (1954)*).

The finding of positive correlations between the intake of high ascorbic acid-containing foods and maternal serum ascorbic acid levels and also between intake of leafy green and yellow vegetables and maternal serum carotenoid levels comes as no surprise since the serum levels of these two substances depend heavily upon the amounts recently ingested. The middle-class white women had higher serum ascorbic acid levels than the subjects from the low income groups.

The finding that serum protein levels were generally higher in women of group A (low income) than in members of group C (middle class) comes as a surprise. Hemoglobin levels did not differ greatly for the two groups. The Selected Group revealed a number of interesting relationships among the chemically determined blood constituents. Among the more consistent of these is the correlation between serum ascorbic acid and serum carotenoid levels of maternal blood, the correlation between serum ascorbic acid of maternal and infant blood, and the correlation between serum carotenoid levels of maternal and infant blood.

Whether the study attains the objective of establishing normality standards for the levels and physiologic changes of the levels of six nutritionally important blood components is open to question. The Selected Group was chosen by such criteria as free-

dom from infectious disease, absence of frank malnutrition, normal clinical course of pregnancy and delivery, etc. The Selected Group did include individuals with substandard dietary ratings. On the other hand, dietary habits were probably temporarily improved (in varying degrees) during pregnancy due to the popular recognition of the nutritional needs of pregnancy. These two factors among others may cause one to question whether normality standards have been established. This is by no means a criticism of the Macy study. It merely points to some of the tremendous technical difficulties involved in the establishment of such standards and might serve notice that more studies of a similar nature are required.

THE EFFECT OF DIETARY VEGETABLE FAT ON THE SERUM LIPIDS

During the past four years L. W. Kinsell and his colleagues have described experiments dealing with the effects of high fat diets on the serum cholesterol and phospholipids of human subjects. This work has been described in several journals (*J. Clin. Endocrinol.* **12,** *909 (1952)*; *J. Clin. Nutrition* **1,** *224 (1953)*; *J. Am. Dietet. Assn.* **30,** *685 (1954)*). These workers contend that either mixed or "chemically constant formula diets" which contain large amounts of vegetable fat lead to prompt reductions of the serum total cholesterol and phospholipid. These effects were said not to occur when similar amounts of animal fat were fed and were said to be reversed when animal fats were substituted for vegetable fats.

The observations of Kinsell *et al.* are worthy of careful study, for the authors propose that vegetable fats contain some factor which tends to lower the serum cholesterol and phospholipid levels. Most other workers have concluded that animal and vegetable fats were interchangeable insofar as their effect upon these serum lipids was concerned (E. A. Hildreth, S. M. Mellinkoff, G. W. Blair, and D. M. Hildreth, *Circulation* **3,** *641 (1951)*; A. Keys, O. Mickelsen, E. v. O. Miller, and C. B. Chapman, *Science* **112,** *79 (1950)*).

Many of the experimental trials of Kinsell *et al.* must be discarded from consideration because of the presence of multiple variables which prevent clear evaluation of the effect of the dietary fat treatment. The authors' remark that "... it should be noted that the formula diets have been emulsified with either a cerebroside preparation, or Tween 80 ..." and "... the effects observed, however, do not appear to depend upon the emulsifier which is used ..." leave the reader uncertain of the importance of this practice to the experiments, for no data are shown to support this conclusion. The "formula diets" appear to have been administered by an in-dwelling gastric catheter. The authors assure that "Excepting only the 'pure fat' diets the patients were maintained in weight equilibrium and excellent nutrition including a positive nitrogen balance" (*J. Clin. Nutrition* **1,** *224 (1953)*). This is a startling statement, for many of the subjects were receiving 60 to 85 per cent of their total calories from fat and were shown to have significant ketonemia. Unfortunately the authors show neither body weight performances nor nitrogen balances, so that this undocumented statement is the only available information.

The serum cholesterol observations are of particular interest in these experiments. They are notable because of the low initial levels recorded and the exceedingly low levels which they often reached with the vegetable fat treatment, these being under 100 mg. per cent in some subjects and under 150 mg. per cent in many others. This degree of hypocholesterolemia is rarely seen

in adult human subjects and then generally only in cachectic or moribund patients. The serum cholesterol reduction was observed to be in large part a reduction of esterified cholesterol with a smaller fall in the full portion.

These changes, a profound hypocholesterolemia, with the reduction particularly of the esterified portion, have been produced by G. V. Mann and H. S. White (*Metabolism* **2,** *47* (*1953*)) in animals subjected to inanition. The effect could be reproduced with ACTH but not with cortisone. These workers also showed that the removal of physiologic stress by the treatment with cortisone of the acute phases of rheumatoid arthritis in human patients would permit the deviated low cholesterol levels to return toward normal. Similar phenomena have been observed by D. Adlersberg, L. E. Schafer, and S. R. Drachman (*J. Clin. Endocrinol.* **11,** *67* (*1951*)) who at first interpreted their findings as indicating a hypercholesterolemic effect of ACTH.

This interpretation of the data of Kinsell *et al.* as reflecting the effect of the stress of the dietary regimen is consonant with the profound intolerance of human subjects to a dietary mixture furnishing more than 60 per cent of the caloric supply as fat. Stimulated by the often repeated statements of polar explorers, the palatability and practicability of a high fat, high protein diet has been repeatedly examined with human subjects (R. M. Kark, R. E. Johnson, and J. S. Lewis, *War Medicine* **7,** *345* (*1945*); *Nutrition Reviews* **3,** *314* (*1945*); F. C. Consolazio and W. H. Forbes, *J. Nutrition* **32,** *195* (*1946*)) and in each instance found unsatisfactory. Nutritionists will doubt the contention of Kinsell *et al.* that subjects receiving 87 per cent of their total calories from fat could be in caloric and nitrogen equilibrium even when exhibiting ketonemia.

Until more conclusive evidence is documented there will be skepticism concerning the statement that vegetable fat in the diet furnishes some component which influences sterol and phospholipid metabolism in the manner described by these authors.

ADVANCES IN THE STUDY OF BODY COMPOSITION

Basic data on the composition of the body are supplied by direct chemical analysis. This provides the final reference point for the indirect methods applicable to normal, undernourished and malnourished human beings. In spite of their fundamental importance, published analytic data on the chemical composition of the adult human body are scant. In recent years there have been only two communications on this subject (H. H. Mitchell, T. S. Hamilton, F. R. Steggerda, and H. W. Bean, *J. Biol. Chem.* **158,** *625* (*1945*); E. M. Widdowson, R. A. McCance, and C. M. Spray, *Clin. Sci.* **10,** *113* (*1951*); *Nutrition Reviews* **10,** *14* (*1952*); **11,** *205* (*1953*)). Consequently, any additional data deserve attention, especially if the subjects studied were relatively normal clinically. This is true of the body analyzed by R. M. Forbes, A. R. Cooper, and H. H. Mitchell (*J. Biol. Chem.* **203,** *359* (*1953*)). The patient was a white male, 46 years of age, definitely underweight (53.8 kg. as compared to the standard weight of 69.5 kg.; relative weight of 77.4 per cent). Death was due to a skull fracture, as a result of a fall, five days after the accident. The postmortem examination revealed congestion of the lungs but no other abnormalities were noted. The patient's appearance was that of a thin man in good physical condition.

Analyses are reported for 13 separate tissues and organs. Here reference will be made principally to the data on the body as a whole. Fat (ether extract) accounted

for 19.7 per cent of the gross body weight. It is of interest that separable adipose tissue represented only 11.4 per cent of body weight. Substantial amounts of fat were contributed by the skeleton and the striated muscles. Their weights represent 17.6 per cent and 39.8 per cent of the total body weight, respectively. The composition of adipose tissue should be noted (ether extract 71.6 per cent, water 23.0 per cent, protein 5.9 per cent, ash 0.2 per cent). In the fat-free body mass, water accounts for 69.4 per cent, ash for 6.8 per cent, protein (nitrogen × 6.25) for 23.4 per cent. Useful comparison is made of the data from the study by Forbes *et al.* (*loc. cit.*) and those reported in the literature.

Among the indirect methods an important role is played by the estimations based on body density and total body water and its fractions. We can speak of a densitometric and a hydrometric approach to body composition.

For the living man, the determination of body volume needed for the calculation of body density has been made on the basis of the Archimedes' principle. On submersion, the body loses in weight an amount equivalent to the weight of the displaced fluid (W). Knowing this value and the temperature of the water (and thus its density, d), we can calculate the displaced volume (V = W/d), equal to the volume of the body. The procedure is unquestionably cumbersome and fairly time-consuming as it demands the determination of the amount of air in the lungs and the respiratory passages at the time of underwater weighing.

Two new approaches to the measurement of body volume have been reported. Both of the reports, concerned with the application of the technic to man, are in the form of short abstracts and a detailed critical evaluation of the procedures is not yet available. R. J. Wedgwood and R. W. Newman (*Am. J. Phys. Anthropol.* **11,** *260 (1953)*) noted that the method for determining body density by immersion of the subjects in water is inapplicable to the very young and the infirm, and proposed an air-displacement (rather than a water-displacement) procedure. The volume of air in the air-tight compartment in which the subject is enclosed is successively increased and decreased by known amounts through the action of bellows, and the changes of pressure within the enclosed space are noted. These pressure changes are recorded electrically and the volume occupied by the body is calculated from calibration data obtained by the use of cylinders of known volume.

In the second approach, developed by W. E. Siri (*Fed. Proc.* **12,** *133 (1953)*) and employing a gas dilution technic, the body volume is obtained from the change in concentration of helium injected into a closed chamber, with and without the subject in the chamber. A thermal conductivity cell serves to measure the helium concentration.

In addition to a new technic for the assessment of body volume, Siri (*loc. cit.*) proposed a method for computing the total body fat from a formula which essentially combines the densitometric and the hydrometric method. The body is divided into three compartments: water, fat and the lean (fat-free) tissue solids. An obvious limitation of the approach is the heterogeneity of the last compartment and the lack of reliable information about its density.

The determination of body volume by submersion requires active cooperation of the subject. It is evident that it cannot be applied to living animals. The animals must be killed and eviscerated in order to determine the volume of water displaced on immersion.

A method for determining the body volume of intact animals by helium dilution, similar to that proposed by Siri (*loc. cit.*), was described in detail by M. Walser and S. N. Stein (*Proc. Soc. Exp. Biol. Med.* **82,** *774 (1953)*).

A carefully measured amount of helium is added to the chamber containing the animal. When the gas has mixed completely with the air in the chamber and in the animal's lungs, a sample of the mixture is analyzed. The volume of the animal is obtained as the difference between the volume of the empty chamber and the volume in which the helium was diluted. The latter volume is obtained as the ratio of the volume of the added gas to the gas concentration in the sample. No correction for residual air, contained in the lungs and the respiratory passage, is necessary. Helium is an inert gas, diffuses rapidly, and its solubility in blood is low (0.0087 cc. per cubic centimeter of water, 0.0148 cc. per cubic centimeter of fat). The amount of helium removed during the time required for complete mixing from the atmosphere surrounding the animal is negligible. By varying the size of the chamber and the amount of helium injected it should be possible to adapt the technic to a wide variety of animal species.

Having determined the total density of a two-component system and knowing the density of the components, one can readily calculate the proportional mass of either component. This principle underlies the estimation of body fat by the densitometric technic. In his pioneering paper, A. R. Behnke (*Harvey Lectures* **37,** *198 (1941/1942)*) has defined these components as lean body mass, associated with a density of 1.099, and "excess fat" with a density of 0.940. This value was obtained experimentally for tissues lost by a man undergoing weight reduction. A. Keys and J. Brozek (*Physiol. Rev.* **33,** *245 (1953)*) gave the density of tissues gained by 10 men in the course of six months of positive calorie balance as 0.948. Thus, within a certain range of weight changes, the tissues gained or lost appear to be of similar density and may be expected to be similar in composition. However, these are complex tissues, including extracellular fluid and nonfat cellular components in addition to chemically defined fat.

Behnke's co-workers, E. N. Rathbun and N. Pace (*J. Biol. Chem.* **158,** *667 (1945)*), in developing a formula for calculating total body fat from body density, used for human fat the density value of 0.918 given in the "Handbook of Chemistry and Physics" (edited by C. D. Hodgman, *28th ed. (1944)*). This value refers to a determination of specific gravity made at 15°C. (as compared with the body temperature of about 36°C.) on fat extracted from subcutaneous adipose tissue of a corpulent man killed in an accident (H. Jaeckle, *Z. physiol. Chem.* **36,** *53 (1902)*). No adequate information existed for adjusting the value for the temperature of the living body.

Impelled by the paucity of information on an important body constant and the need for exact knowledge of the density of body fat for the densitometric estimation of the fat content of the living human and animal body, F. Fidanza, A. Keys, and J. T. Anderson (*J. Applied Physiol.* **6,** *252 (1953)*) undertook a study of the density of body fat extracted from adipose tissues of man and various laboratory and domestic animals. The samples of human fat were obtained at surgery, those of animals shortly after death. For man the mean density based on twenty determinations was 0.9000 g. per cubic centimeter at 37°C., with trivial differences between 5 individuals (3 males, 2 females) and between locations. The standard deviation was 0.0007. Effect of temperature on the expansion of fat was examined in the range from 10° to 37°C. At 36°C. the density is 0.9007 g. per cubic centimeter. At 15°C. a density of 0.9167 (specific gravity of 0.9175) was found for human fat, a value which closely agrees with Jaeckle's specific gravity of 0.9179.

The density of animal fats was similar or somewhat higher (maximum of 0.9212 for internal fat of lamb). No significant differences were found in the internal and subcutaneous fat in man, the dog, rat, rabbit

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The density of animal fats was similar or somewhat higher (maximum of 0.9212 for internal fat of lamb). No significant differences were found in the internal and subcutaneous fat in man, the dog, rat, rabbit

and guinea pig, while such differences were found in the steer, pig and lamb. The thermal expansion in the range of 15° to 37°C. is about four times larger for human fat, and as much as seven times larger for some animal fats than the expansion of water. The density value of fats obtained at room temperature cannot be applied, without correction for temperature, to living organisms.

MAGNESIUM DEFICIENCY IN CALVES

It is known that magnesium is required in the diet of vertebrates for growth, reproduction and prevention of various deficiency signs including hyperirritability and convulsions (*Nutrition Reviews* **7,** *198 (1949)*). A recent review on magnesium metabolism in cattle emphasizes the importance of an adequate intake of this mineral by cattle on pasture for the prevention of "grass tetany" (*Ibid.* **12,** *214 (1954)*). Because of the practical nature of the problem, basic studies on magnesium deficiency are especially important.

An interesting study on magnesium deficiency in calves has been made by K. L. Blaxter, J. A. F. Rook, and A. M. MacDonald (*J. Comp. Path.* **64,** *157 (1954)*) and by Blaxter and Rook (*Ibid.* **64,** *176 (1954)*). This work presents additional clinical and pathologic findings.

The magnesium-low basal diet simulated milk and contained casein, glucose and margarine as the chief components. It was supplemented with adequate amounts of reagent grade minerals (except magnesium), vitamins, peanut oil and lecithin. This was made up to one liter with water. The basal diet contained only 0.35 to 0.50 mg. of magnesium per 100 ml. Seven Ayrshire bull calves weighing 30 to 35 kg. were used in the experiments.

The first signs of deficiency seen in the calves fed the basal ration were changes in the carriage of the head and ears. Opisthotonus (head retraction) similar to that seen in thiamine deficiency was observed in all calves. All calves were hypersensitive, as shown by twitching of the skin and an extreme apprehensive condition. Later the calves developed fine tremors of the musculature, frothing at the mouth, and a slight rise in body temperature and pulse rate. In the final stages of the deficiency, five to seven weeks from the beginning of the experiment, severe convulsions appeared, resulting in death. Two control calves fed 19 mg. of magnesium per 100 ml. of diet remained normal throughout the experiment.

Very little change was seen in body weight in the deficient calves until severe clinical signs developed. The deficiency was associated with a fall in blood serum magnesium to values below 0.5 mg. per cent unless at least 14 mg. of magnesium were fed per 100 ml. of milk. Signs of tetany were not observed until the blood serum magnesium had fallen to below 0.7 mg. per cent. Blood calcium and phosphorus levels remained normal. No detectable loss of magnesium from the soft tissues of the body was noticed, but bone magnesium was depleted by about 30 per cent.

On postmortem examination deficient calves were found to have hemorrhagic areas, particularly in the heart, in the large blood vessels, and in the abdominal cavity including the kidney. Histologic examination showed thrombus formation in the small venules of the pericardial fat associated with necrosis of the wall, as well as other changes in the heart. No significant lesions were seen in the muscles or in the central nervous system.

It was concluded that the growing calf required a dietary concentration of 16 to 18 mg. of magnesium per 100 ml. of milk for

normal performance and maintenance of tissue levels. These values are lower than those suggested by C. F. Huffman, C. L. Conley, C. C. Lightfoot, and C. W. Duncan (*J. Nutrition* **22,** *609 (1941)*), but they are still considerably higher than the magnesium content of normal cow's milk (7 to 10 mg. per 100 ml.). The results show that when whole milk is the sole diet for the calf magnesium retention is limited. This suggests that in the future more attention should be given to magnesium in nutrition experiments dealing with growing animals fed whole milk diets.

It is possible that signs of deficiency in calves would be different in older animals or in animals kept on diets with suboptimal levels of magnesium over extended periods. However, basic studies of the type reviewed here are essential for a proper understanding of the role and importance of magnesium in nutrition and physiology. It appears that the practical importance of this element has not been fully recognized in the past.

CHLORELLA AS HUMAN FOOD

Considerable interest is being maintained in the possible future mass production and processing of the algae *Chlorella* as a food for animals and man. Reviews on the industrial and economical culture of *Chlorella* and on its composition have recently appeared (*Nutrition Reviews* **9,** *347 (1951)*; **11,** *77 (1953)*; **12,** *111 (1954)*). According to F. J. Weiss (*Scientific American* **187,** *No. 6, 15 (1952)*), millions of people throughout the world have eaten seaweed as a food in various forms since ancient times. Even today alginic acid, prepared from algae, is used in large amounts as a stabilizer by the food industry. However, until now little direct experimental work has been conducted on the possible practical use and acceptance of dried *Chlorella* cells in modern foods.

An interesting preliminary study by Y. Morimura and N. Tamiya of the Tokugawa Institute for Biological Research, Tokyo, Japan, in cooperation with the Carnegie Institute of Washington (*Food Technology* **8,** *179 (1954)*) demonstrates that palatable human foods can be made containing varying amounts of *Chlorella* powder. The authors used a product consisting of dried cells of *Chlorella ellipsoidea*, which is green in the powdered form. This product was used to enrich the following foods in the amounts indicated in parentheses: French bread (5.9 per cent), rolls (10 per cent of the flour), noodles (2 to 6 per cent), Japanese-style green tea (up to 20 per cent), and ice cream (9.9 per cent). The foods prepared in this manner were said to be "tasty" and "agreeable" as determined by several Japanese and American adults. Such foods also had what was described by the authors as an "agreeable" light green color.

The effect of additions of *Chlorella* on the nutrient composition of the prepared foods was calculated. Significant amounts of protein, fat, minerals and certain vitamins were contributed by the *Chlorella* cells. Especially noteworthy were the large amounts of vitamin A and ascorbic acid in the food products, particularly in the bread and noodles. The *Chlorella* cells themselves contained 5000 I.U. of vitamin A (as carotene) and 2 to 5 mg. of ascorbic acid per gram of dry weight by direct analysis.

Various soups, both Japanese and western styles, as well as a substitute for soy sauce, were also successfully prepared using *Chlorella* cells.

This interesting study is admittedly preliminary and further experiments are needed on the acceptability and nutritive value of such foods. Larger taste panels than used in this study would be required. It is doubtful, for instance, that Americans would

accept green-colored bread or rolls for routine use unless through necessity. Also needed are direct nutrient analyses of the final food products rather than calculated analyses.

In spite of the limitations, experiments such as these are important because of the promise they offer for the stretching of future world food supplies. Whether such foods would be accepted will no doubt depend largely on economic conditions and changing food habits in the future.

THIAMINE LOSSES AND SALT MIXTURES

In a previous review (*Nutrition Reviews* **10,** *134* (*1952*)) the marked instability of thiamine in certain purified diets was emphasized. The occurrence of an apparently new deficiency syndrome on diets to which 0.2 mg. per cent of thiamine had been added was shown to be curable by thiamine and to be due to the rapid destruction of the vitamin on storage. A. A. Kandutsch and C. A. Baumann (*J. Nutrition* **49,** *209* (*1953*)) later presented extensive studies upon the rate of thiamine destruction in a typical purified diet containing Wesson salts. At room temperature only 21 per cent of the thiamine remained after one week. The stability was considerably better in the refrigerator, but about half was lost in one month. The authors showed that unsaturated fats exerted a deleterious effect while ascorbic acid, hydroquinone, cysteine, or sulfasuxidine had a protective action. The best stabilizing agent found was glycerol added as 1 per cent of the diet. In contrast to crystalline thiamine, the thiamine in yeast and dried pork when added to the diet was found to be completely stable.

A more recent paper from the same laboratory (P. E. Waibel, H. R. Bird, and C. A. Baumann, *J. Nutrition* **52,** *273* (*1954*)) is concerned with the effects of the constituents of the salt mixtures upon thiamine stability. The thiamine content of a purified diet of typical composition was determined at intervals after preparation with various components of the salt mixture added singly or in combinations. Diets with a complete salt mixture (G. M. Briggs, T. D. Luckey, C. A. Elvehjem, and E. B. Hart, *J. Biol. Chem.* **148,** *163* (*1943*)) promoted thiamine destruction. Only 17 per cent of the original thiamine remained after three days' storage at 37°C. and only 76 per cent was recoverable when the diet was analyzed immediately after preparation. On the other hand, 92 and 93 per cent were found for the above respective conditions when the diet contained no salt mixture. The activity of the salts was related to the fineness to which they were ground. Much more destruction occurred when they were prepared by grinding in a ball mill than when they were simply mixed together in a mixer. From studies with the individual salts used in the preparation of the mixture it appeared that di-potassium phosphate was largely responsible for the destructive action. Calcium carbonate and manganous sulfate accelerated the destruction to a lesser degree, but di-potassium phosphate alone produced as much loss as the complete salt mixture.

Presumably the action of di-potassium phosphate is related to its alkalinity and hygroscopic nature. Five g. of the salt exposed upon a watch glass in the laboratory gained over 0.1 g. of water in forty-five minutes and in twenty hours became completely liquid. Di-sodium phosphate is nondestructive, although it is as alkaline as the potassium salt, but is much less hygroscopic and less soluble. A salt mixture containing tricalcium phosphate and potassium chloride to supply the calcium, potassium, and phosphorus supplied by di-potassium phosphate and calcium carbonate in the salt mixture used had no deleterious effect upon thiamine. Other

recent papers have implicated salt mixtures as being responsible for the destruction of vitamins (J. E. Rombouts, *J. Nutrition* **50,** *255* (*1953*); R. L. Lyman and C. A. Elvehjem, *Proc. Soc. Exp. Biol. Med.* **77,** *813* (*1951*)). In both cases the salt mixture contained di-potassium phosphate. The USP mixture (U. S. Pharmacopoeia, *Ed. XI, p. 479* (*1936*)) and that proposed by J. H. Jones and C. Foster (*J. Nutrition* **24,** *245* (*1942*)) do not contain this salt and thiamine is apparently relatively stable in diets containing them (J. A. Reyniers *et al.*, *Ibid.* **41,** *31* (*1950*)).

These studies emphasize not only the need for a reappraisal of some current salt mixtures but also the need for continual care when any modification of a diet is made. These data emphasize the role of modifications in the salt mixture, but it is possible that various other apparently innocuous changes may produce profound changes in the diet or the performance of animals receiving them.

GOLDTHIOGLUCOSE OBESITY

Obesity continues to be a public health problem of interest, since it correlates with the incidence of certain "degenerative diseases" and with longevity (*Nutrition Reviews* **11,** *144, 292* (*1953*)). Obesity results from discrepancy in caloric balance, namely, excess caloric intake in relation to expenditure. Although all the factors responsible for the control of caloric balance are not known, several experimental approaches to the study of obesity have been made. A marked increase in food intake and obesity result from experimentally produced hypothalamic lesions in several mammalian species (*Ibid.* **2,** *105* (*1944*)). The relation of the increased food intake which follows these lesions to alterations in blood sugar levels and to the "glucostatic theory" of regulation of food intake has been discussed previously (*Ibid.* **10,** *280* (*1952*); **11,** *55* (*1953*)).

Two other biologic situations offer approaches to the study of the factors involved in the etiology of obesity and in the long-term consequences of overnutrition. The first of these is a hereditary obesity which occurs in a certain strain of mice (*Nutrition Reviews* **10,** *303* (*1952*)). These mice consume more calories and fat but less protein and carbohydrate than nonobese control mice of the same strain offered the same diets. The oxygen consumption of the obese mice is about half that of the nonobese whether calculated per unit of weight or in relation to surface area. Fasting respiratory quotients of obese and control mice are comparable. Hereditarily obese mice are further characterized by the presence of hyperglycemia and glycosuria, refractoriness to insulin, and sensitivity to growth hormone, pancreatectomy, and adrenocorticotropic hormone. Metabolic study has further demonstrated that obese mice oxidize a smaller proportion of administered acetate than do control mice, and that unoxidized acetate is recovered as body fat, suggesting a metabolic derangement resulting in increased lipogenesis (*Ibid.* **11,** *88* (*1953*)).

Secondly, G. Brecher and S. H. Waxler (*Proc. Soc. Exp. Biol. Med.* **70,** *498* (*1949*)) reported that obesity results and persists in mice given a single intramuscular or intraperitoneal injection of goldthioglucose. Near lethal doses of the gold salt are necessary to produce this effect. Approximately one third of the mice which survived the injection demonstrated marked increases in body weight which were due largely to increased amounts of adipose tissue as determined by carcass analyses. A centrilobular fatty infiltration of the liver was noted on microscopic examination, but no other abnormalities which might explain the experimentally produced obesity were

demonstrated in any other organ. Waxler and Brecher (*Am. J. Physiol.* **162,** *428 (1950)*) further demonstrated that the obesity was associated with increased food consumption by the treated mice during periods of development and maintenance of obesity, or periods of refeeding after starvation. Obese animals when starved mobilized their excess body fat, and quickly regained their prestarvation weights after refeeding by overeating.

A recent report concerns the mechanism of goldthioglucose obesity in mice, and some adaptive responses of mice to chronic overnutrition (R. H. Drachman and J. Tepperman, *Yale J. Biol. Med.* **26,** *394 (1954)*). The effect of fasting and of diet upon the induction of goldthioglucose obesity in mice was studied. Survival rate and the incidence of obesity among survivors were increased in mice which were fasted for twenty-four hours before and for twenty-two hours after the injection of 1 mg. of goldthioglucose in oil per gram of body weight. A tendency for more extreme degrees of obesity to occur among the fasted mice was also apparent.

In another experiment mice were maintained for six weeks on isocaloric diets providing 21 per cent of total calories either as carbohydrate (glucose, sucrose) or as fat (lard) and then injected intraperitoneally with goldthioglucose. Survival rates in these groups of experimental animals were 40 per cent and 17 per cent, respectively. Fat feeding and starvation differed with regard to their effect upon the susceptibility of mice to goldthioglucose toxicity. The results observed during starvation could not be attributed to a predominance of fat metabolism which occurred when the mice were deprived of food.

The effect of goldthioglucose at different levels of blood glucose was also tested. Two groups of mice were fasted for twenty-four hours. One group was given glucose intraperitoneally fifteen minutes prior to the injection of the gold salt, and the other group received only the gold salt. During a four-month eriod fewer of the mice given glucose survived as compared to the control group. Although the results of the above studies do not provide definitive interpretation, it is of interest that the toxicity of goldthioglucose in mice was affected by alterations in the diet.

Whether or not the obesity-producing ability of goldthioglucose could be prevented by dimercaptopropanol (BAL), a drug which protects against toxicity of several heavy metals, was determined. Injections of BAL resulted in an increased incidence of survival and a concomitant decrease in the incidence of obesity. The toxic potency of the gold salt was not separable from its obesity-producing qualities in these experiments.

The adaptive responses of mice to long-established (maximum of five months) obesity induced by goldthioglucose were observed. The obese mice did not develop high blood sugar levels or abnormalities in glucose tolerance, or hypersensitivity to the hyperglycemic effect of a growth hormone preparation. Liver fat content and serum cholesterol were significantly increased in the obese mice as compared to controls, findings similar to those noted previously in hypothalamic obese rats (J. R. Brobeck, J. Tepperman, and C. N. H. Long, *Yale J. Biol. Med.* **15,** *831 (1942/1943)*). Absolute oxygen consumption was 79 per cent greater in the obese mice, but when calculated as a function of surface area there was no difference between the obese mice and control mice. Oxygen consumption of liver slices obtained from obese mice and from control mice, calculated per milligram of nitrogen, was also comparable. The liver, heart and kidneys of the obese mice weighed 60 to 80 per cent more than the viscera of control animals. No obvious microscopic differences were noted in the hearts and

kidneys of the two groups. The "hypermetabolism" of the obese mice was attributed to the enlargement of the viscera and increase in "active metabolic mass" which resulted as an adaptive response to obesity. Renal lesions and ovarian atrophy which occur in rats with hypothalamic obesity were not noted in mice made obese with goldthioglucose.

A comparison of some metabolic characteristics of hereditarily obese mice of one strain and of mice of another strain with goldthioglucose obesity has been reported by J. Mayer and C. Y. Zighera (*Science* **119,** *96* (*1954*)). Swiss mice made obese with goldthioglucose had normal blood sugar levels which were not altered by the administration of growth hormone, and displayed sensitivity to insulin. Hereditarily obese mice developed hyperglycemia, responded to growth hormone with increases in blood sugar levels, and were insensitive to insulin. Nonobese littermates of hereditarily obese mice were made obese with goldthioglucose. These mice had normal blood glucose levels, displayed no response to growth hormone, and were insulin sensitive. These findings indicated that the metabolic differences noted in comparing the genetically obese and the chemically obese animals were not attributable to a characteristic of the strain of mice studied. This study is particularly noteworthy since two types of obesity, each with its distinct metabolic pattern, were produced in mice of the same litter. Further studies utilizing this favorable experimental situation to inquire further into other metabolic aspects of these different types of obesity and their exact etiologies will be awaited with interest.

Hypothalamic obesity, hereditary obesity of mice, and goldthioglucose obesity of mice present certain points of similarity and of difference. In none of these are the exact anatomic lesions, biochemical mechanisms, or possible alterations in metabolic pathways associated with the obesity fully understood. Studies designed to elucidate more clearly the points of similarity and of difference involved in these situations may provide useful information concerning the factors involved in the regulation of food intake, the development of obesity, and the adaptive responses of the organism to obesity. Careful study of such biologic or pharmacologic oddities may give rise to facts pertinent to a better understanding of the important problem of human obesity and its consequences.

DIETARY FACTORS AND RADIATION DAMAGE

The study of dietary factors which may prevent or minimize damage from radiation injury is of current interest. Evidence suggesting that liver might contain hitherto unidentified substances which are protective against the effects of chronic x-irradiation in rats has been presented (*Nutrition Reviews* **11,** *13* (*1953*)).

A series of papers has appeared from Bowman Gray School of Medicine describing the influence of diet on survival following administration of P^{32} to mice. After a short period on an experimental diet, usually five days, mice were given 4.5 to 6.0 microcuries of P^{32} intraperitoneally per gram of body weight. The time required for 50 per cent of the mice to die, the per cent of the mice living after twenty-one days, and the average survival time were used as indexes in the evaluation of the various diets.

The effect of dietary protein, fat, lipotropic agents and phosphate was the basis of the first report (W. E. Cornatzer, G. T. Harrell, D. Cayer, and C. Artom, *Proc. Soc. Exp.*

Biol. Med. **73,** *492 (1950)*). It was found that fatty infiltration of the liver produced either by carbon tetrachloride administration or by choline deficiency did not alter the twenty-one-day survival of mice given P^{32}. A protective action was observed with a diet containing 10 per cent protein and 5 per cent fat. A high protein, high fat diet had a detrimental effect on survival.

Further studies have evaluated the influence of B-vitamin deficiency. The addition of folacin or folacin plus vitamin B_{12} had no beneficial effect on the survival of mice receiving 6 microcuries of P^{32} (W. E. Cornatzer, C. Artom, G. T. Harrell, and D. Cayer, *Proc. Soc. Exp. Biol. Med.* **76,** *552 (1951)*). When the dose of radiophosphate was reduced to 5 microcuries and the formation of these vitamins in the gut was inhibited by sulfasuxidine, it was possible to demonstrate that the addition of relatively generous amounts of folacin and vitamin B_{12} had a definite beneficial effect whereas little improvement was observed with either vitamin alone.

The protective role of pyridoxine was investigated (C. Artom, W. E. Cornatzer, and G. T. Harrell, *Proc. Soc. Exp. Biol. Med.* **79,** *494 (1952)*) because a therapeutic action of this vitamin had been observed in mice following whole body x-irradiation (A. Goldfeder, L. Cohen, C. Miller, and M. Singer, *Ibid.* **67,** *272 (1948)*). Pyridoxine did not mitigate the lethal action of P^{32} on mice fed a diet with 5 per cent casein as the protein source. In the control animals fed the same diet which did not contain pyridoxine no manifestations of pyridoxine deficiency became evident. Following induction of pyridoxine deficiency by feeding desoxypyridoxine or by raising the protein content of the pyridoxine-deficient diet to 30 per cent and adding cystine and methionine, pyridoxine had a beneficial effect.

The influence of pantothenic acid and its analogues on radiation injury from P^{32} is the subject of the latest report from this laboratory (C. Artom, *Proc. Soc. Exp. Biol. Med.* **86,** *162 (1954)*). The addition of pantothenic acid to a pantothenic acid-deficient diet had a slight protective action in lowering the mortality following P^{32}. The addition of sulfasuxidine to the diet made it possible to show a definite therapeutic effect with pantothenic acid. Para-aminobenzoic acid was without significant influence on the mortality. The substitution of pantoyltaurine for pantothenic acid proved just as effective as pantothenic acid. The effect of this sulfur-containing analogue of pantothenic acid was apparently not due to its sulfur content because free taurine conferred only slight benefit. Another analogue of pantothenic acid, methyl pantothenic acid, was without significant influence.

This series of investigations suggests that pantothenic acid, pyridoxine, and the combination of folacin and vitamin B_{12} are active in delaying the lethal action of P^{32} in mice. The administration of choline, vitamin K, biotin, para-aminobenzoic acid and alpha-tocopherol was not beneficial. The degree to which the observed results were specific to the stress of radiation cannot be considered established. The question whether these observations apply to other types of radiation remains unanswered. It is evident that the comparison of these results with those of other workers using different diets, species and conditions of radiation is hazardous. In commenting on the potential clinical significance of these observations the authors conclude that, "in the radiation syndrome, diminished food intake, impaired absorption from the intestine, severe damage to other tissues, and, in addition, administration of large amounts of antibiotics might lead to an actual deficiency. In such a condition there would be a definite indication for the administration of generous amounts of those vitamins which, in our experiments, appeared to exert a beneficial action."

RIBOFLAVIN AND THE STRESS RESPONSE

When animals are subjected to a variety of stimuli such as exposure to cold, heat, or high altitude there is a clear-cut physiologic response. This so-called stress response requires the presence of a functioning adrenal cortex. The biochemical manifestations of the stress response have been studied in great detail and include depletion of adrenal ascorbic acid, increased excretion of 17-ketosteroids, and an increased rate of gluconeogenesis.

Of particular interest to the nutritionist is the possible relationship of various nutrients to the stress response. It has been pointed out (B. H. Ershoff, *Physiol. Rev.* **28,** *107 (1948)*) that the requirements for various nutrients may be increased during the stress condition. Since the same stress response is obtained to a wide variety of stimuli these considerations assume practical importance, as for example in the design of combat rations for troops in cold climates.

The recent report of B. R. Forker and A. F. Morgan (*J. Biol. Chem.* **209,** *303 (1954)*) clearly demonstrates the importance of riboflavin in the stress response. These workers exposed rats to low oxygen tension as the stress factor and used the response of liver glycogen in the fasted animal as the criterion of response. An increase in liver glycogen in a fasted animal reflects gluconeogenesis, which is a reliable biochemical measure of the magnitude of the stress response. Weanling Long-Evans rats were given a purified diet with and without riboflavin. Growth was normal when rats were given the supplemented diet whereas rats receiving the diet without riboflavin failed to grow after the first week of feeding. Animals subjected to anoxia were placed in a specially designed chamber with oxygen tension to simulate 20,000 feet altitude. The animals were fasted during the twenty-four-hour period they remained in the chamber and were then killed and the livers analyzed for glycogen. These glycogen levels were compared to those of rats which were fasted at sea level oxygen tensions.

The first experiment was designed to study the effect of time on the riboflavin-deficient diet on the response to anoxia. It was found that after approximately three weeks of feeding the animals receiving the deficient diet gave essentially no gluconeogenic response to anoxia. In contrast animals receiving riboflavin exhibited approximately a ten-fold increase in liver glycogen in response to anoxia.

In a second experiment a study was made of the effect of adrenal hormones and of a single injection of riboflavin on the response to anoxia. The deficient animals again failed to respond. The injection of 0.1 mg. of riboflavin into the deficient animals immediately before the test resulted in complete restoration of the gluconeogenic response to the stress. Also, the administration of adrenal cortical extract or cortisone to the riboflavin-deficient animals completely restored their ability to respond to anoxia. Desoxycorticosterone was ineffective in this respect. A group of rats receiving the complete diet was adrenalectomized and they failed to respond to the anoxia.

These experiments show that the adrenal gland or its hormones are necessary for the gluconeogenic response to anoxia since adrenalectomized animals failed to respond even though fed a complete diet. Also, since the riboflavin-deficient animals would respond to cortisone or complete cortical extract, the riboflavin effect is not due to a failure of the target tissues to respond to the hormones. It may be concluded that riboflavin is required either for synthesis of the adrenal hormones or else it functions in the mechanism concerned with their elaboration. The fact that riboflavin was effective when injected into the deficient animals immediately before the test indicates that the vitamin may function in a direct manner. This

is in contrast to the situation with pantothenic acid, a deficiency of which also impairs the gluconeogenic response to anoxia. In the case of pantothenic acid, injection of the vitamin immediately before the test does not result in a gluconeogenic response to the stress (L. S. Hurley and A. F. Morgan, *J. Biol. Chem.* **195,** *583 (1952)*).

DIET AND HEPARIN IN EXPERIMENTAL ATHEROSCLEROSIS

Although atherosclerosis experimentally induced in animals is not necessarily related to that occurring in man, investigations of this nature can be expected to contribute to the investigation of the human disease. Several recent contributions have been made by inducing the disease in chicks, rabbits and hamsters (*Nutrition Reviews* **9,** *295 (1951)*; **10,** *148 (1952)*).

Atherosclerosis has been particularly difficult to induce in rats (*Nutrition Reviews* **11,** *152 (1953)*). The contribution of R. W. Wissler, M. L. Eilert, M. A. Schroeder, and L. Cohen (*Arch. Path.* **57,** *333 (1954)*) is particularly welcome since their experiments were conducted with carefully controlled conditions. In this study 136 "middle-aged," obese male rats were given a semipurified diet similar in carbohydrate, fat and protein content to the average diet of 4 patients with coronary thrombosis who were between 35 and 50 years of age. Ten of the animals were killed immediately before the study was begun and no disease was found. These animals and all others selected for the various regimens were randomly chosen. The remaining 126 rats were placed in the six groups of 21 each to which various diets were supplied. To one group 25 mg. of thiouracil was administered daily. Four of the 6 diets furnished 6 g. of fat daily; the 2 other diets provided 2 g. of fat daily. Two groups were given 125 mg. of choline daily; the remainder received 12.5 mg. of choline daily. All but one of the groups were given 62.5 mg. of added cholesterol per day. At the end of fourteen weeks 3 animals of each group were killed. At this time no lesions were found in the arteries.

At twenty weeks 6 of the remaining animals in each group were subjected to a hypertensive regimen, consisting of the administration of rabbit antirat-kidney serum for two days. This was followed a week later by daily subcutaneous injections of desoxycorticosterone acetate and 1 per cent sodium chloride for drinking water. The remainder of the animals served as controls for the hypertensive regimen and received plain water for drinking, no serum injections, and sesame oil instead of the hormone. The animals were continued on these regimens for twenty more weeks or until they died (10 died between twenty-seven and thirty-six weeks and 19 between thirty-seven and forty-four weeks).

The animals not on the hypertensive regimen continued gradually to gain weight throughout the study, except for those receiving thiouracil. In the latter there was a drop in body weight at first, which was mitigated by a change in dosage. The animals on the hypertensive regimen steadily declined in body weight soon after the antiserum was administered. Some animals lost as much as 250 g. The authors state that this loss of weight was not accompanied by a diminished food consumption and they postulated increased nitrogen loss in the urine to account for it. Nitrogen balance was not studied.

There was some rise in blood pressure in a few of the rats not on the hypertensive regimen, but a striking increase was observed in almost all of the animals given the treatment. Thus, after forty-five days of treatment only 2 of the 55 controls had a systolic blood pressure above 150 while 23 of 27 animals on the hypertensive regimen were above this value.

Histopathologically, after administration of some of the experimental rations lipid was deposited in the walls of many of the coronary arteries, less so in the renal arteries and the aorta. The lesions were described as of two types, "lipomatous," with fatty deposits but without proliferative changes, and "atheromatous," in which fatty changes and proliferation of the cellular elements of the arterial walls were found. In addition some lipid was found deposited in the glomerular tufts of the kidneys, particularly those from the animals rendered hypertensive. Arteritis (inflammation of an artery) was found, particularly in the parenchymatous organs, but its significance could not be determined, as it occurred in animals on other diets. The extent of lipomatous and atheromatous changes in the arteries was determined by examining them grossly and microscopically.

An attempt was made to correlate the lesions with the 5 different diets, with thiouracil administration, and with the hypertensive regimen. With respect to the latter, 54 per cent of those to which the regimen was applied developed lesions, whereas only 22 per cent of those given the diet alone developed the characteristic changes. Wide variations in the incidence of lesions on the different diets makes interpretation of the influence of these agents difficult. In the first place thiouracil seemed to have little, if any, effect. Secondly, a high incidence of lesions in the nonhypertensive animals receiving choline on the higher fat diet was found, whereas on the same diet none of the hypertensive animals showed the characteristic arterial changes. When cholesterol was omitted from the diet, the nonhypertensive animals showed no lesions whatsoever, but 100 per cent of the hypertensive animals did. The maintenance of obesity appeared to bear no relation to the occurrence of arterial lesions.

Serum cholesterol and lipid phosphorus levels were measured in these animals and it was found that there was a tendency either for an increase in serum cholesterol or in lipid phosphorus in the groups with an increased incidence of lesions, but the correlation was not significant. However, an increase in the cholesterol/lipid phosphorus ratio was significantly correlated with the presence of coronary disease in particular.

The authors stressed this last correlation, and the fact that these animals were old and obese before the study was begun and that they were fed the experimental diet longer than usual. There did not appear to be a preventive action of choline upon the genesis of the arterial lesions.

The effect of choline upon the development of atherosclerosis in various animal species and in man has been the subject of considerable debate and as noted above was not established in rats. Further work on this subject has been carried out by G. L. Duff and G. F. Meissner (*Arch. Path.* **57,** *329 (1954)*) who have reported four studies involving 81 young adult white rabbits fed commercial rabbit food. Cholesterol was added to the diet by dissolving it in ether, mixing the solution with the food and allowing the ether to evaporate completely. For the administration of choline 1 g. was dissolved in 4 ml. of water and mixed with the food. Daily measurements were made of food consumption and the animals were pair-fed. At the termination of the experiment the animals were killed and the extent of atherosclerosis was determined visually after staining with Sudan IV, and chemically by measuring the quantity of free and total cholesterol. Both of these determinations were done on the aorta. Lipid phosphorus and neutral fat were also determined in the blood.

In the first experiment 14 animals were given 1 g. of cholesterol daily for ninety-three days. Eight of these also received 1 g. of choline daily. At the end of the study there were increases in body weight, and in free and total cholesterol, lipid phosphorus and neutral fat in the serum, but the extent of these increases and the extent of athero-

sclerosis were the same in the animals with or without added dietary choline. In the second study 9 of 18 animals were given 2 g. of choline daily. The results were identical to those in the first study. The third study consisted of 26 animals 13 of which received 2 g. of choline daily. This time the animals were killed in pairs and there appeared to be somewhat less atherosclerosis in the animals given choline, but the differences were not considered significant. The final study involved the feeding of 1 g. of cholesterol daily for three months to 23 animals. At the end of three months the cholesterol feeding was stopped and the normal diet was resumed. A week later 11 were given 3 g. of choline daily for five months. Then all of the animals were killed. Again, the animals treated with choline appeared to have a slight reduction in atherosclerosis, but it was not significant. All other measurements were identical in the two groups. The authors concluded from this study that choline in the doses given did not inhibit the development nor promote the regression of cholesterol atherosclerosis in rabbits.

Further studies on atherosclerosis in rabbits involved the effect of heparin on the serum lipids and on the development of atherosclerosis, carried out by L. Horlick and G. L. Duff (*Arch. Path.* **57**, *417*, *495* (*1954*)). The first of these studies was designed to determine whether heparin would inhibit the formation of atherosclerotic lesions and the rise in serum lipids in rabbits fed cholesterol. First, 39 unselected white rabbits, 3 to 6 months of age, were divided into two groups. All were given a diet containing 1 g. of cholesterol in 8 ml. of corn oil mixed with Purina rabbit pellets, daily for twelve weeks. One group was given intramuscular heparin daily and the other received placebos. The heparin was given in a menstruum and was sufficient to prolong blood clotting time for from eight and one-half to twenty hours. Grossly there was less atherosclerosis in the animals given heparin and also total and free cholesterol in the aortas was considerably less, the total decreasing from 25 to 9 mg. per 100 g. and the free from 9 to 3.4 mg. per 100 g. There appeared to be some drop in serum lipids, but again it was not significant in the heparinized animals.

In order to refine the experiment the authors studied 30 pairs of pedigreed littermates of the same sex. Eighteen of the pairs survived. Again there was generally less atherosclerosis in the animals treated with heparin, but this was not always the case. That is, 11 pairs of controls had lesions which were more extensive than the heparin-treated mates, but the reverse was true in 2 pairs. Confirmatory evidence of the effect of heparin was found in the cholesterol content of the aortas. Generally, the controls of each of the pairs showed higher serum lipids than the animals treated with heparin, but there was no real difference when the animals were considered as two groups. The authors believe that this demonstrates that heparin did not have a significant quantitative effect upon the serum lipids. They concluded that the large doses of heparin decreased or retarded the formation of atherosclerosis in the aorta in these cholesterol-fed rabbits, and that the effect was not due to a quantitative change in the serum lipids, but perhaps to a qualitative modification thereof.

The second study with respect to heparin was upon the regression of atherosclerosis. In this instance the studies were much the same as those noted above except that heparin was given after cholesterol feeding had been discontinued and its effect upon the rate of regression of the lesions was analyzed. In this case, heparin appeared to have no effect upon the rate of regression of the lesions or upon the return of the serum lipids to normal (*see Nutrition Reviews* **12**, *294* (*1954*)).

These studies taken together would indicate first that a vascular disease may be induced in old, obese rats by prolonged cholesterol feeding and is particularly

marked when hypertension is induced at the same time. Secondly, choline had little if any effect upon the genesis of atherosclerosis in rabbits, whereas larger subcutaneous doses of heparin, although not influencing the serum lipids quantitatively, did reduce the degree and extent of the atherosclerotic lesions.

FRUCTOSE OR GLUCOSE FOR THE DIABETIC

Evidence has accumulated over the past few years that the sugars glucose and fructose are prepared for combustion in different ways although the major parts of their oxidative breakdown are identical. Glucose adds a phosphate group at its number 6 carbon atom, and some initial reaction in the utilization of glucose appears to be modified by hormones of the anterior pituitary and the adrenal cortex as well as by insulin. The principal digestion product of the food carbohydrates is glucose. Since the diabetic subject produces too little insulin, a major portion of his available energy source enters the oxidative breakdown with difficulty. On the other hand fructose, when it begins its metabolic breakdown, adds the phosphate group at the position 1 carbon atom, a process which appears to be independent of insulin. Tissues taken from diabetic animals have been shown to burn fructose at a normal rate although their ability to burn glucose is diminished. These observations have renewed the interest in fructose as a dietary carbohydrate for the diabetic (*see also Nutrition Reviews* **12,** *227 (1954)*).

H. P. Sarett and L. P. Snipper (*J. Nutrition* **52,** *525 (1954)*) have compared glucose and fructose as dietary carbohydrates for rats made diabetic by the injection of alloxan. Weanling male rats were maintained on a ration containing 18 per cent casein, 10 per cent corn oil and 64 per cent dextrinized starch. After four weeks on this regimen, they were fasted and injected with a solution of alloxan. Frequent blood sugar determinations were carried out to test the effectiveness of the alloxan injection.

In the first experiment some rats were transferred to the glucose-containing diet and some to a fructose-containing diet three weeks after the injection of alloxan. After four weeks the diets of the two groups were interchanged so that each could serve as its own control. In the second experiment the rats were maintained on the original dextrin-containing diet for three months after alloxan injections. They were then changed to either a glucose- or a fructose-containing diet. In a third experiment the animals were transferred to fructose-, glucose-, or dextrin-containing diets immediately after the injection of alloxan and maintained on these rations for seven months before being killed.

Under all these conditions weight gains, blood sugar levels and food intake were the same on glucose- and on fructose-containing diets; however, water intake, urine volume and urinary carbohydrate excretion were considerably smaller when fructose was fed. Although the growth rates of the diabetic animals were less than those of normal controls, carcass composition did not differ greatly between normal and diabetic rats. The latter contained somewhat less lipid and a somewhat greater amount of water. Liver glycogen was appreciably higher in the diabetic group. The kidneys of the diabetic rats were larger than those of control animals. Diabetic rats receiving the fructose diet contained somewhat more carcass fat and had larger livers than did the glucose-fed diabetic animals. Diabetic rats continued on the dextrin-containing diet showed much lower blood sugar levels than did the diabetic rats fed either the glucose or the fructose diet. The diminished excretion of sugar in the urine of fructose-fed animals may be an

indication that this sugar is taken up by the tissues more readily than glucose, especially in the diabetic animal. Such an interpretation of the results is supported by the studies carried out with isolated tissues taken from diabetic animals.

VITAMIN B_T, CARNITINE

Vitamin B_T, the growth factor required by the yellow meal worm, *Tenebrio molitor* (G. Fraenkel, M. Blewett, and M. Coles, *Nature* **161,** *981* (*1948*)), was isolated and characterized as carnitine by H. E. Carter, P. K. Bhattacharyya, K. R. Weidman, and Fraenkel (*Arch. Biochem. Biophys.* **38,** *405* (*1952*)). Concentrates prepared by previously described methods (Fraenkel, *Ibid.* **34,** *468* (*1951*)) were further purified by chromatography on alumina columns and distribution by the Craig countercurrent method between phenol and dilute hydrochloric acid and yielded crystalline material. This material was shown to be carnitine and to be active for the meal worm. Carnitine is the betaine of beta-hydroxy-gamma-amino butyric acid and has long been known to be a constituent of muscle. Meat extracts contain 1.5 to 3 per cent carnitine. It is of interest that hydroxy-amino butyric acid itself has some carnitine activity for *Tenebrio molitor*, thus suggesting that the larvae can methylate this material to form the betaine.

The assay developed by G. Fraenkel (*Arch. Biochem. Biophys.* **34,** *457* (*1951*)) has been used to study the distribution of carnitine in various materials (Fraenkel, *Biol. Bull.* **104,** *359* (*1953*)). The *Tenebrio* larvae are grown upon a "purified" diet similar to those used for rats. It contains casein, glucose, salt, cholesterol, thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, choline chloride, inositol, folacin and biotin. On this diet the larvae grow slowly and begin to die in three to four weeks. With 0.35 micrograms of carnitine per gram of diet the larvae grow at a steady rate and survive. Faster growth is obtained with higher levels, and it appears that approximately 1.5 micrograms per gram of diet permits maximum growth, about 60 mg. per larva at 9 weeks of age. Materials to be assayed are ground or homogenized and incorporated in the diet and growth of larvae fed these diets is compared to that of controls receiving known levels of carnitine, also mixed in the diet. When homogenization was difficult, water extracts were made and added to the diet.

Several microorganisms, *Escherichia coli*, *Tetrahymena geleii*, and *Streptococcus hemolyticus*, were assayed. The first two, grown upon synthetic mediums devoid of carnitine, contained very little carnitine, less than 1 microgram per gram of solids. The *S. hemolyticus* cells contained about 28 micrograms per gram, but since these were not grown upon synthetic mediums the value may not be significant. On the other hand, brewers' yeast from several sources and *Neurospora* contained considerable carnitine, 8 to 35 micrograms per gram of solids. Vegetable materials appear to vary widely in carnitine activity. Wheat assayed 7 to 14 micrograms per gram of solids, whereas corn contained less than detectable amounts. Spray-dried alfalfa juice gave a value of 20 micrograms per gram, whereas similar preparations from young rye and oat plants contained very little, if any.

The carnitine content of *Tenebrio* larvae grown on various levels of carnitine was studied. None was detectable in those which were reared upon the deficient diet. Larvae grown upon diets containing various amounts of carnitine indicated that 50 per cent or more of the carnitine consumed is retained in bodies of the larvae. Similar studies done with the larder beetle larvae, *Dermestes vulpinus*, which does not require

carnitine in the diet showed a high content in the bodies regardless of whether it was supplied in the diet or not. The larvae of the blowfly, *Phormia regina*, also contained considerable carnitine when they were grown upon a synthetic diet. Thus at least two insect species which do not require the vitamin in the diet are able to synthesize it efficiently while *Tenebrio* has no capacity for its synthesis.

The carnitine content of the developing hen's egg was studied. No detectable activity was found in the egg whereas the embryo contained considerable activity. Practically all of the carnitine in the chick is produced by synthesis. Various mammalian tissues were assayed. Skeletal muscle is by far the most active (560 to 1120 micrograms per gram), with other tissues showing variable and lesser amounts. There appeared to be no particular difference among dog, rat, and rabbit tissues insofar as they were studied. The urinary excretion by human subjects was dependent upon the diet. The highest values were obtained following a heavy meat meal and after a three-day period of starvation (132 to 264 micrograms per milliliter). Low values of 14 to 56 micrograms per milliliter were found when the diet consisted largely of fruits and vegetables. The output is thus apparently raised either by the consumption of large amounts or by the breakdown of body tissue during starvation.

The widespread occurrence of carnitine in vegetable and animal tissues would suggest an important metabolic role. The author points out that whereas the small amount required by *Tenebrio* is suggestive of a catalytic function, the large amount in muscle suggests perhaps another role.

It is not clear where this factor fits into the picture of the vitamin T-complex previously discussed (*Nutrition Reviews* **9,** *19 (1951)*). The similarity of the designation of these two growth factors is purely accidental. Fraenkel states that vitamin T preparations have only a very weak vitamin B_T action, and therefore, they cannot be identical as suggested by J. Leclercq (*Arch. internat. de Physiol.* **57,** *350 (1950)*). It has also been shown that "Vitamin T," a crude extract from *Torula* yeast, contains a multitude of factors (W. Goetsch, *Oesterr. zool. Zeitschr.* **3,** *140 (1951)*; A. Wacker, H. Dellweg, and E. Rowold, *Klin. Wchnschr.* **29,** *780 (1951)*).

SOME FUNCTIONS OF DIETARY FAT

The simple concept that dietary fats serve merely as convenient sources of calories and as carriers of fat-soluble vitamins has not been tenable for some time. Fats have been shown to stimulate caloric intake, increase growth rate and physical performance, improve reproduction and lactation, and have other effects. A series of recent papers has extended the list of special functions of fats or lipids.

Study of the relative merits of butterfat and other nutritionally important fats continues with a paper by T. Viswanatha, J. E. Gander, and I. E. Liener (*J. Nutrition* **52,** *613 (1954)*). These authors concerned themselves with the relation of vitamin E and the carbohydrates sucrose and lactose to the growth-promoting properties of butterfat as compared with corn oil. Weanling rats were fed a ration containing 20 per cent casein, 48 per cent carbohydrate (either lactose or sucrose), 28 per cent fat (either corn oil or butterfat), and 4 per cent salt mixture. The control group received alpha-tocopherol by daily oral administration. In the initial experiment the authors employed young rats from mothers of unknown dietary history. In the absence of vitamin E and with the use of lactose as dietary carbohydrate, weight gain was

appreciably better with butterfat than with corn oil. In the presence of vitamin E, the two fats showed approximately equal growth-promoting ability. With sucrose as the dietary carbohydrate, the two fats were equally effective in promoting growth, regardless of the presence or absence of vitamin E. Subsequent efforts to demonstrate the superiority of butterfat on diets containing lactose but lacking vitamin E failed. The authors therefore conducted their experiments with younger animals which had been depleted of vitamin E.

The experimental animals were offspring of mothers receiving a vitamin E-deficient diet from the time of parturition. Only those animals showing definite symptoms of vitamin E-deficiency were selected. The criteria employed were subnormal weaning weight, muscular paralysis of the hind legs, and hemolysis of the red blood cells by dialuric acid. Under these conditions, the superiority of butterfat on lactose-containing, vitamin E-deficient rations could again be demonstrated with statistically significant results. In order to accentuate the vitamin E deficiency, the authors included TCP (tri-o-cresyl phosphate) in the rations of young weanling rats from mothers raised on ordinary stock rations. The diet also contained 2 per cent sulfathalidine.

The young were maintained on the TCP-containing diet for two weeks. At this time the animals grew only slowly, showed a rough hair coat, diarrhea and a positive hemolysis test. Under these conditions the superiority of butterfat on a lactose-containing ration devoid of vitamin E was again demonstrable. A somewhat less significant difference between butterfat and corn oil was also demonstrable on the sucrose-containing diet. The addition of vitamin E to the ration did not significantly improve the weight gains observed on any of the vitamin E-deficient rations. One is led to wonder, therefore, whether TCP and sulfathalidine did under the conditions employed in these experiments actually aggravate the vitamin E deficiency or whether these substances act in some other manner. In any case, a statistically significant superiority of butterfat over corn oil was demonstrated on the vitamin E-supplemented ration with either lactose or sucrose as the dietary carbohydrate and containing TCP and sulfathalidine. The inclusion of 2 per cent sulfathalidine alone in a vitamin E-containing ration also made possible the demonstration of butterfat's superiority over corn oil on either lactose or sucrose diets.

In another experiment the authors determined the effect of corn oil and of hydrogenated vegetable oil on the alpha-tocopherol level of blood serum. The hydrogenated vegetable oil supported virtually identical serum tocopherol levels when either lactose or sucrose was employed as the dietary carbohydrate. On the other hand corn oil supported normal levels of serum tocopherol only in the presence of sucrose. With lactose as the dietary carbohydrate, corn oil feeding led to a marked lowering of the blood vitamin E level. This small experiment suggests that the combination of corn oil and lactose in the diet, particularly a vitamin E-deficient diet, was detrimental. It also leads one to wonder whether butterfat is superior to corn oil or whether corn oil is inferior to other commonly available fats. The authors themselves speculate on the possibility that lactose together with the unsaturated fatty acids from corn oil accelerate the oxidative destruction of vitamin E in the alimentary tract. It is unfortunate that the authors interpret their sulfathalidine experiment in terms of a modified intestinal flora. While this is certainly one possible explanation, it is clear from the paper that no investigation of the intestinal flora was carried out. Unless such experiments are done, it is well to exercise considerable caution when invoking the effect of intestinal bacteria.

The protective action of dietary fats in x-irradiation has been further explored by

A. L. S. Cheng, M. Ryan, R. Alfin-Slater, and H. J. Deuel (*J. Nutrition* **52,** *637 (1954)*). Deuel, Cheng, G. D. Kryder, and M. E. Bingemann (*Science* **117,** *254 (1953)*) have previously reported on the protective action of methyl linoleate against x-irradiation.

In the present study young male rats were fed a fat-free ration for periods of nine to twelve weeks, at which time they ceased to grow and showed symptoms of essential fatty acid deficiency. They were then divided into four groups, group 1 serving as control, group 2 receiving 10 mg. of methyl linoleate per day, group 3 receiving 50 mg. per day and group 4, 100 mg. per day. All four groups were continued on the original fat-free ration. Supplementation was continued for eight weeks. After this, x-irradiation was begun at the rate of 200 roentgens per week for ten weeks. Throughout the irradiation period and for three weeks thereafter the animals continued to receive the fat-free ration as well as the linoleate supplement. The unsupplemented controls showed an average survival time of 50.7 days, 50 per cent of the animals being dead fifty-four days after the beginning of irradiation. The total average radiation dose was 1453 roentgens. None of the animals survived the thirteen weeks following the beginning of irradiation. Rats receiving 10 mg. of linoleate per day showed a survival time of 71.5 days, 50 per cent of the animals being dead seventy-three days after the start of irradiation; total exposure was 1826 roentgens, with one-third of the animals surviving the experiment. Considerably better protection was offered by the 50 and 100 mg. doses of linoleate. In these cases more than two-thirds of the animals survived the experimental period. Why linoleate should protect against the effects of ionizing radiation is not clear.

The effect of potassium, sodium, or calcium salts added to a purified diet fed to young growing rabbits has been studied by J. G. Wooley and O. Mickelsen (*J. Nutrition* **52,** *591 (1954)*). The basal diet contained either 20 or 30 per cent casein and from 1 to 12 per cent fat. The diet was essentially complete, already containing the minerals provided by the inclusion of 4 per cent Wesson salt mixture. Increasing the fat content of the 20 per cent casein basal diet led to a diminution in growth response. On the other hand, if the basal diet was supplemented with sodium, potassium, or calcium salts, increasing the fat content of the ration led to a moderate increase in weight gain. This effect was still more pronounced on the 30 per cent casein diet supplemented with additional salts. The addition of sodium salt was as effective as supplementation with all three minerals. Added individually, sodium and calcium supplementation were more effective than the addition of potassium salts. It seems that the rabbit, at least, has somewhat unusual mineral requirements which still require further investigation, and that these mineral requirements determine in part the animal's response to varying levels of dietary fat.

Interesting interrelationships among steroids have been revealed in a study by W. T. Beher and W. L. Anthony (*J. Nutrition* **52,** *519 (1954)*). These workers concerned themselves particularly with the fatty livers produced by cholesterol feeding. Seven groups of young adult male mice were fed a basal ration consisting of 25 per cent casein, 55 per cent sucrose, 16 per cent nonnutritive material and 4 per cent salt mixture. Vitamins E and K, members of the B-complex, and para-aminobenzoic acid, inositol and ascorbic acid were added to the ration. On the other hand vitamins A and D were omitted. The addition of 1 per cent cholesterol and 0.25 per cent cholic acid to the basal ration more than doubled the cholesterol content of the liver. Greater concentrations of cholic acid fed along with 1 per cent cholesterol increased still further the cholesterol content of the liver. On the other hand adding dihydrocholesterol in 2.5 per cent concentration along with

cholesterol and cholic acid drastically reduced the cholesterol level of the liver. When the mice were fed rations containing 1 per cent cholesterol, 2.5 per cent dihydrocholesterol and different concentrations of cholic acid, it was found that increasing the cholic acid content of the ration decreased the total cholesterol of the liver. The analytic results obtained suggest that dihydrocholesterol was not deposited in the liver. It may be that compounds such as dihydrocholesterol and soybean sitosterol (which has a similar action) inhibit the absorption of dietary cholesterol.

A. E. Hansen, J. G. Sinclair, and H. F. Wiese (*J. Nutrition* **52,** *541 (1954)*) have reported on the sequence of histologic events in skin during the onset of fat deficiency. They reported distinct and characteristic alterations in the stratum corneum, in the collagen structure, in the dermis and in associated structures such as hair follicles, sebaceous (sebum-producing) glands and sudoriparous (sweat-producing) glands. The observed changes appeared to be due primarily to a deficiency in the essential unsaturated fatty acids.

F. H. Mattson, J. H. Benedict, and L. W. Beck (*J. Nutrition* **52,** *575 (1954)*) have extended their earlier work on the mechanism of absorption of fat from the alimentary tract. In the 1930's Verzár set forth a theory of fat absorption which was quite generally accepted for many years. According to this theory fats are digested by pancreatic and intestinal lipases, breaking the triglyceride into the component glycerol and three molecules of fatty acids. The water-soluble glycerol was presumably absorbed directly, passing into the cells lining the small intestine. The fatty acids were rendered more or less water-soluble by loose combination with bile salts. The fatty acid-bile salt complex was thought to pass into the lining of the intestine. The bile salts then returned to the liver, while the fatty acids recombined with glycerol to re-form the fat molecule, which then passed into the lymphatic circulation. The 1940's were dominated by Frazer's partition theory, according to which undigested fat could be absorbed and passed directly into the lymphatics, while such fatty acids as might be split from some of the fat would be absorbed into the portal circulation. According to Frazer, solubilization of fats is accomplished by mixtures of fatty acids, bile salts and monoglycerides (partial digestion products of fat). At the present time the mood is one of uncertainty and general disagreement.

The study of Mattson, Benedict and Beck deals with the nature of the digestion products of fat and the composition of the material in the intestines during fat digestion and absorption. Rats fasted for forty-eight hours were given cottonseed oil by stomach tube. They were killed after three hours, and the intestinal contents were removed for analysis. Of the fatty material remaining in the small intestine, somewhat more than 60 per cent was triglyceride (or undigested fat) plus diglyceride (fat from which one fatty acid had been removed). Roughly 10 per cent was present as monoglyceride, and about 25 per cent as the free fatty acid. When synthetic tricaprin was administered in place of cottonseed oil, more than 70 per cent of the intestinal content consisted of triglyceride plus diglyceride, while only 16 per cent existed as free fatty acid. This suggests that shorter chain fatty acids such as capric acid are absorbed more rapidly than the longer chain acids of a fat such as cottonseed oil.

Unfortunately, the authors give no information about the total amount of lipid which disappeared during the three-hour experimental period, yet such information is vital if one is to speak of absorption rates. When cottonseed oil was fed together with a 1-monoglyceride (the fatty acid attached to an end carbon of glycerol), the concentration of the several intestinal lipids was the same as when only cottonseed oil had been administered. Similar constancy of composi-

tion was noted when cottonseed oil was fed together with a 2-monoglyceride, with the fatty acid attached to the center carbon of glycerol. Only when very large amounts of the 2-monoglyceride were fed with cottonseed oil did the composition of intestinal lipids differ from that observed with the feeding of cottonseed oil alone. Under these conditions somewhat larger concentrations of 2-monoglyceride remained in the intestines. When cottonseed oil was fed together with free fatty acids, the 2-monoglyceride usually found in the intestinal contents disappeared almost completely, while the concentration of free fatty acids increased particularly when very large amounts of fatty acids were administered.

While these experiments demonstrate the remarkable ability of the alimentary tract to maintain a constant medium from which fat absorption takes place, they do not permit choice of one of the several theories of fat absorption proposed in recent years. Nevertheless, it is information of this type which is very much needed before a new theory of fat absorption can be formulated.

MINERALS AND DENTAL CARIES PREVENTION

It has been observed that Norway rats raised on a laboratory stock diet during the period of tooth development are more resistant to dental caries than rats raised on a purified diet adequate in known essential nutrients (*Nutrition Reviews* **6,** *196 (1948)*). One of the differences between the two diets employed was that the natural stock diet contained 6.5 per cent of minerals whereas the purified diet contained 4.0 per cent of reagent salts.

Workers at the Harvard School of Dental Medicine have recently investigated whether substitution of the ash from the natural diet for the reagent salts in the purified diet would influence caries development in Norway rats (R. F. Sognnaes and J. H. Shaw, *J. Nutrition* **53,** *195 (1954)*). Ash was prepared from the natural diet as follows: The diet was preheated in large porcelain trays in an oven at 130°C. for twenty-four hours and was then placed in a muffle furnace for twenty-four hours at 550°C. By this procedure about 7.0 per cent of the diet was recovered as ash.

At the beginning of the experiment adult female rats were maintained prior to and during first litter pregnancy and lactation on the purified diet containing 4.0 per cent of reagent minerals. Their first litters were fed on this diet during the entire caries-evaluation period. During the second litter gestation and lactation period, the mothers were fed the ash diet (purified diet in which the 4.0 per cent of reagent minerals was replaced by 4 per cent of the ash prepared from the natural diet). At weaning time the young were returned to the purified diet containing reagent salts. The mothers were continued on the ash-containing diet through a third complete reproductive cycle. The third-litter young were fed the purified diet after weaning. In the fourth complete reproductive cycle the ash-containing diet was used throughout. Each litter was killed seven months after birth. Thus, the adult female rats were fed the purified diet through the first complete reproductive cycle and up to mating in the second reproductive cycle while the young were subjected to varying regimens ranging from complete purified to complete ash diet during the stages of gestation, lactation and the postweaning period.

Replacement of reagent salts of the purified diet with ash from the natural diet significantly lowered the incidence of carious lesions in the young. At 7 months of age the 13 young that had a dietary history of ash diet throughout the experimental period averaged 1.4 carious lesions per animal while 47 that had a dietary history of puri-

fied diet throughout gestation, lactation and the postweaning period averaged 6.2 carious lesions. The 50 animals with a history of ash diet during gestation or lactation, followed by purified diet during post-weaning, averaged 4.2 carious lesions. Included in the experiment were parallel litters of young, under the various dietary treatments, that were desalivated (all major salivary glands removed) at the time of weaning. In such animals caries induction was overwhelming (21 or more carious lesions per animal) regardless of dietary history.

These results indicated that considerable, although not complete, protection against dental caries in Norway rats could be accomplished through inclusion in a purified diet of the ash from a protective natural diet. This is of importance since in earlier studies it was noted that a 50 per cent increase in the amount of reagent salts in the purified diet failed to bring about a reduction in caries incidence (J. H. Shaw, *Proc. Soc. Exp. Biol. Med.* **70,** *479 (1949)*).

The known effects of fluorine as a caries-preventive factor were recognized and it was noted that the purified diet contained 2.5 parts per million (p.p.m.) of this element while the ash diet contained 6 p.p.m. A considerable portion of the fluorine was lost in ashing since the natural stock diet was reported to contain 21 p.p.m. (*Nutrition Reviews, loc. cit.*).

In another experiment the Harvard workers investigated the influence of supplementing the purified diet with 6 or 25 p.p.m. of fluorine as sodium fluoride (J H. Shaw and R. F. Sognnaes, *J. Nutrition* **53,** *207 (1954)*). Litters of young exhibited almost identical carious lesions whether raised and maintained throughout the experiment on the purified diet alone or whether they received a supplement of 6 p.p.m. of fluorine during tooth development, after tooth development, or both. Although supplementation with 25 p.p.m. of fluorine during lactation lowered the caries incidence, the effect was not significant. Only when the 25 p.p.m. fluorine supplement was used throughout gestation and lactation was a significant reduction in the caries incidence noted (decrease from 9.3 to 4.8 in average number of lesions per animal).

These results should not be interpreted as being in conflict with the effectiveness of fluorine in caries inhibition in children where it has been established that 1 to 2 p.p.m. of fluorine in the drinking water produces a reduction of 40 to 60 per cent in the dental caries incidence. In the present studies with rats the purified diet employed probably has vastly greater caries-producing potential than the average human diet. In addition, much more fluorine has been shown to be required by rats to achieve the degree of caries protection observed in children by 1 to 2 p.p.m. in the drinking water (H. C. Hodge and R. F. Sognnaes, "Dental Caries and Fluorine," *p. 53, American Association for the Advancement of Science, Washington, D. C. (1946)*). The results nevertheless indicate that the real protection against caries, obtained from inclusion in the purified-type rat diet of the ash from a natural diet, cannot be explained on the basis of increased fluorine intake.

Spectrographic analyses of the ash from the natural diet revealed qualitatively the presence of at least 10 trace elements that are thus far not considered essential nutrients for animals. Whether or not any of these, rather than possibly a more optimum ratio of known elements, is responsible for better caries protection on diets containing ash than on diets containing reagent salts remains to be determined. The authors have considered both possibilities and cite a recent reference which suggests that vanadium, when fed postdevelopmentally, possesses a measurable caries-inhibiting effect in Syrian hamsters (C. F. Geyer, *J. Dent. Res.* **32,** *590 (1953)*).

These results should serve as a stimulus toward extending our present limited evidence regarding the relation of trace elements, other than fluorine, to tooth decay.

NOTES

Letter to the Editor

Dear Sir:

Numerous statements have appeared to the effect that the diet of the Taiwanese living in Formosa contains less than 300 mg. of calcium daily. During the months of May and June, and again in September and October (1954), extensive clinical nutritional surveys were done on these people. There was no evidence of calcium deficiency demonstrable clinically. A thorough investigation was made as to a possible source of calcium outside of that contained in the food.

This calcium was located in the rice consumed by these people. The rice milling in Formosa is accomplished by numerous small local units. The millers have a practice of adding what is known locally as "stone powder" to the rice during the milling process. The "stone powder" was found to be essentially calcium carbonate. It is added for the purpose of facilitating the flow of the rice kernels through the mills as well as artificially whitening the undermilled rice. This calcium is worked into the rice kernel and remains there even after thorough washing. It is estimated that a kilogram of cooked rice presents from 500 to 1000 mg. of calcium.

There is one drawback to the procedure which is at present being thoroughly evaluated. The presence of the lime salts increases the pH of the cooking water, at times to as high as 9.0. The average cooking time of rice is thirty minutes at the temperature of boiling water, and at this pH some destruction of riboflavin and thiamine can be anticipated.

HERBERT POLLACK, M.D.
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American Board of Nutrition

The American Board of Nutrition will hold the next certifying examinations for Specialists in Human Nutrition during April 1955, at locations convenient for candidates. Completed applications of persons who wish to be considered for certification should be in the office of the Secretary not later than February 1, 1955. Application forms may be obtained from the Secretary, Otto A. Bessey, Department of Biochemistry and Nutrition, The University of Texas School of Medicine, Galveston, Texas.

CLINICAL NUTRITION BRIEFS

"The cardiac output in cirrhosis of the liver is frequently high, and may rise slightly immediately after paracentesis, at a time when hemoconcentration occurs. Rigid dietary restriction of sodium will prevent the reaccumulation of ascites and edema in patients with cirrhosis of the liver. Prolonged dietary sodium restriction is often associated with striking improvement in nutritional status." (*Nutrition Reviews* **12,** *292 (1954)*, Sodium Restriction and Circulatory Dynamics in Cirrhosis.)

"Treatment of these patients with either vitamin B_{12} or folacin was followed by evidence of correction of the anemia. There was an increase in the number of red blood cells, a decrease in the per cent of megaloblasts in the bone marrow, and an increase in circulating reticulocytes. Concurrent with and often preceding these evidences of hematologic response there were changes in the nucleic acids in the bone marrow." (*Nutrition Reviews* **12,** *293 (1954)*, An Effect of Vitamin B_{12} and Folacin on the Nucleic Acid Content of Bone Marrow.)

"Although the mechanism of the heparin-induced lipemia clearing reaction has not been fully elucidated, it appears likely that heparin in conjunction with a factor from plasma forms a complex (clearing factor) which has lipase or lipase-activating properties. A tissue factor may activate the forma-

tion of this clearing factor. The latter substance in the presence of plasma albumin causes clearing of plasma lipemia, redistribution of lipoproteins, and liberation of fatty acids. The albumin probably functions by binding the liberated fatty acids. The pertinence of these interesting studies to the problem of human atherosclerosis is by no means clear." (*Nutrition Reviews* **12,** *294 (1954)*, Heparin and the Lipemia Clearing Reaction.)

"Of some interest also are data on aging, even though the group studied is very small and heterogeneous, containing both men and women. The elderly patients tend to have a higher fat content (31.7 per cent of body weight on the average). The extracellular water compartment was larger (26.5 per cent) than in younger adults. Intracellular water was low (23 per cent as compared with 32 per cent in the younger group). The nonfat cell solids in these two groups were estimated as 13 and 16 per cent, respectively." (*Nutrition Reviews* **12,** *324 (1954)*, Body Water and Body Composition.)

"A diet high in fat and cholesterol, low in sulfur amino acids and adequate in choline produced marked hypercholesterolemia, elevation of the serum lipoproteins of the S_f 12 to 20 category and typical aortic atheromata. The degree of protein inadequacy was not sufficient to produce any change in total serum protein. The abnormalities in serum lipids and lipoproteins in these monkeys on the amino acid-deficient diet were prevented by inclusion of casein at a level of 25 per cent in place of the deficient protein, and minimized by decreases in total dietary fat or addition of DL-methionine to the deficient diet. The use of diet as a preventive or therapeutic tool in the management of atherosclerosis and its complications is suggested by the researches thus far accomplished." (*Nutrition Reviews* **12,** *325 (1954)*, The Role of Diet in the Management of Atherosclerosis.)

"Five of these patients were given a single injection of 24 mg. of synthetic citrovorum factor (leucovorin). There was a typical blood response in all patients. The red cell count and reticulocytes increased and the mean cell volume decreased. Bone marrow studies demonstrated a change from megaloblastic to normoblastic marrow following the injection. This improvement lasted approximately two weeks, after which time the blood picture began to revert to the original condition of macrocytic anemia. It appears that a single dose of citrovorum factor is effective for approximately two weeks in the treatment of nutritional macrocytic anemia." (*Nutrition Reviews* **12,** *331 (1954)*, Treatment of Nutritional Macrocytic Anemia.)

"The relationships among nutrition, growth and development are complex. Genetic endowment provides the limits within which nutritional factors can operate and the endocrine system is importantly involved. The x-ray analysis makes it possible (1) to distinguish the poorly from the adequately mineralized skeleton, and (2) to judge skeletal age from the presence or absence of ossification centers and epiphyseal fusions, as a useful measure of general body maturity. Thus it provides an important research tool and a useful supplement to the clinical evaluation of nutritional status." (*Nutrition Reviews* **12,** *332 (1954)*, Human Growth.)

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